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# The antimicrobial activity of mononuclear ruthenium(II) complexes containing the dppz ligand

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## Dedication

In memory of Leone Spiccia

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## Abstract

The *cis*- $\alpha$  isomer of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  {dppz = dipyrdo[3,2-*a*:2',3'-*c*]phenazine;  $\text{bb}_7$  = bis[4(4'-methyl-2,2'-bipyridyl)]-1,7-alkane} has been synthesised. The minimum inhibitory concentrations and the minimum bactericidal concentrations of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  and its parent complex  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  (phen = 1,10-phenanthroline) were determined against a range of bacteria. The results showed that both ruthenium complexes exhibited good activity against Gram-positive bacteria, but  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  showed at least eight-times better activity across the Gram-negative bacteria than  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ . Luminescence assays demonstrated that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  accumulated in a Gram-negative bacterium to the same degree as in a Gram-positive species, while assays with liposomes showed that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  interacted more strongly with membranes compared to the parent  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  complex. The DNA binding affinity for  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  was determined to be  $6.7 \times 10^6 \text{ M}^{-1}$ . Although more toxic to eukaryotic cells than  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ ,  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  exhibited greater activity against bacteria than eukaryotic cells.

## Introduction

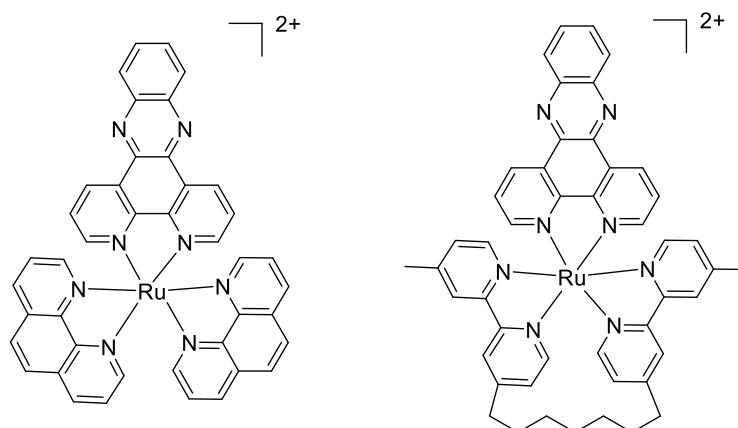
There has been recent interest in developing kinetically-inert polypyridylruthenium(II) complexes as therapeutic agents,<sup>[1-5]</sup> with complexes containing the dipyrdo[3,2-*a*:2',3'-*c*]phenazine (dppz) ligand attracting particular attention.<sup>[6-8]</sup> Barton and co-workers established that  $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$  (bpy = 2,2'-bipyridine) and  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  (phen = 1,10-phenanthroline) strongly bind DNA by intercalation, and because these complexes exhibit negligible background emission in aqueous solution but display strong luminescence upon DNA binding, they are now known as “molecular light-switches”.<sup>[9,10]</sup> The dppz complexes have subsequently been utilised in numerous biological studies; for example, as DNA and cellular imaging agents, nuclear acid-based sensors and as potential therapeutic drugs.<sup>[6-8,11-13]</sup>

Although  $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$  and  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  bind DNA strongly, they have generally exhibited low cytotoxicity against eukaryotic cells, with a number of studies indicating that the low cytotoxicity was due to their poor cellular uptake.<sup>[14-16]</sup> Puckett and Barton demonstrated that these complexes enter eukaryotic cells by passive diffusion, and that the uptake can be enhanced by increasing the lipophilicity of the complex, e.g. by substituting the bpy or phen ligands with 4,7-diphenyl-1,10-phenanthroline (DIP).<sup>[14]</sup> Similarly, a number of more recent studies have also demonstrated that dppz-based complexes which are more lipophilic than  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  exhibit good activity against a range of cancer cell lines: e.g.  $[\text{Ru}(\text{dppz})_2(\text{CppH})]^{2+}$  {CppH = 2-(2'-pyridyl)pyrimidine-4-carboxylic acid}<sup>[17,18]</sup> and



Given the greater presence of negatively-charged components (phospholipids, such as phosphatidyl-glycerol, teichoic acids and lipopolysaccharides) in the bacterial membrane and cell wall compared to eukaryotic membranes,<sup>[20]</sup> it could be anticipated that  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  would show good antimicrobial activity. Consistent with this proposal, Aldrich-Wright and co-workers found that  $[\text{Ru}(2,9\text{-Me}_2\text{phen})_2(\text{dppz})]^{2+}$  (2,9-Me<sub>2</sub>phen = 2,9-dimethyl-1,10-phenanthroline) displayed good activity against Gram-positive bacteria – even drug-resistant strains.<sup>[21]</sup> However, and consistent with other studies, the dppz-based ruthenium complex showed no activity against Gram-negative bacteria. As Gram-negative bacteria have two membrane layers rather than the single cytoplasmic membrane present in Gram-positive species, the poor activity is again due to the lack of cellular uptake.

In order to increase the antimicrobial activity of  $[\text{Ru}(2,9\text{-Me}_2\text{phen})_2(\text{dppz})]^{2+}$ , the 2,9-Me<sub>2</sub>phen ligands could be replaced with a more lipophilic ligand such as DIP. However, this would also significantly increase the toxicity of the ruthenium complex to eukaryotic systems.<sup>[15-17]</sup> In addition, a number of studies have also demonstrated that the cellular localisation of polypyridylruthenium(II) complexes in eukaryotic cells is significantly modulated by the lipophilicity.<sup>[22]</sup> Consequently, it could be beneficial to increase the uptake into bacterial cells through another mechanism that does not require an increase in the lipophilicity. In a recent solid-state NMR and molecular dynamics study of the interaction of the dinuclear complex  $[\{\text{Ru}(\text{phen})_2\}_2(\mu\text{-bb}_{12})]^{4+}$  {where  $\text{bb}_{12}$  = bis[4(4'-methyl-2,2'-bipyridyl)]-1,12-dodecane} with model membranes it was shown that the two metal centres could be embedded at the level of the membrane phosphate group with the alkyl linker dipped into the hydrophobic interior of the membrane.<sup>[23]</sup> As an alternative approach to utilising dinuclear complexes, the alkyl chain could be incorporated into a mononuclear complex through an additional chelate ring involving the polymethylene chain in a tetradendate  $\text{bb}_n$  ligand (where  $n$  = the number of carbons in the chain), as shown in Figure 1. We have previously synthesised  $[\text{Ru}(\text{phen}')(\text{bb}_7)]^{2+}$  (where  $\text{phen}'$  = phen and its 5-nitro-, 4,7-dimethyl- and 3,4,7,8-tetramethyl- derivatives) complexes and examined their antimicrobial activities.<sup>[24]</sup> As the  $\log P$  (a measure of lipophilicity) values for the  $[\text{Ru}(\text{phen}')(\text{bb}_7)]^{2+}$  complexes (-1.33 to -1.83) were approximately the same as that reported for  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  (-1.48),<sup>[14]</sup> we sought to synthesise  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  (see Figure 1) and examine its antimicrobial activity against a range of bacteria, including a number of Gram-negative species.



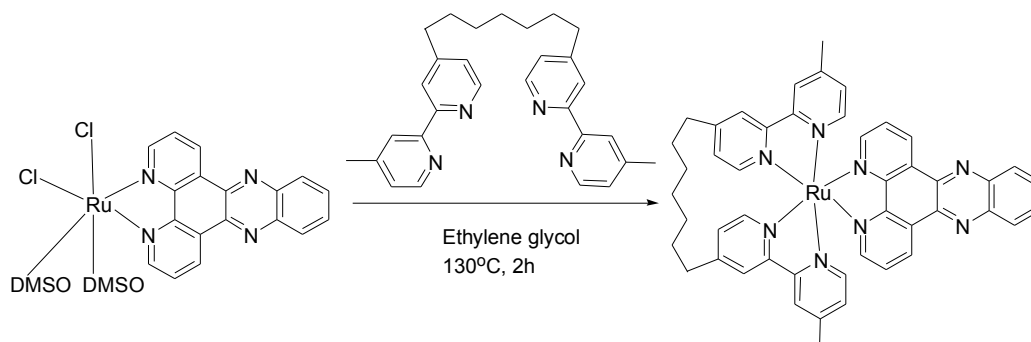
**Figure 1.**  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  (left) and  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  (right)

Herein, we describe the synthesis of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  and an analysis of its log  $P$ , DNA binding and antimicrobial activities compared to  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ . The results demonstrate that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  binds DNA with a slightly higher affinity than  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ , and despite having a very similar log  $P$  value, shows significantly greater activity against Gram-negative bacteria. Furthermore, experiments with liposomes indicate that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  interacts differently with membranes than  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ , while cellular uptake experiments suggest that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  does readily accumulate in Gram-negative bacteria.

## Results

### Synthesis and characterisation

The synthesis of the  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  complex containing the flexible  $\text{bb}_7$  ligand is presented in Scheme 1. In order to avoid the formation of the dinuclear complex, the  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  was synthesised by direct reaction of  $\text{bb}_7$  with the appropriate mole ratio of the precursor complex *cis,cis*- $[\text{RuCl}_2(\text{DMSO})_2(\text{dppz})]$  in ethylene glycol as previously reported.<sup>[25]</sup> The  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  complex was purified by SP Sephadex C-25 cation-exchange chromatography. Only the *cis-α* isomer of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  was obtained, consistent with what was reported for the  $[\text{Ru}(\text{phen}')(\text{bb}_7)]^{2+}$  complexes.<sup>[24]</sup> The  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  complex was characterised by microanalysis, NMR spectroscopy and high-resolution electrospray ionisation (ESI) mass spectrometry. The  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  complex displayed a resolvable  $^1\text{H}$  NMR spectrum, and all proton chemical shifts were assigned based on the comparison with those of similar compounds.<sup>[25,26]</sup> Only one symmetrical set of resonances was observed for both the dppz and  $\text{bb}_7$  aromatic protons.



**Scheme 1** Synthesis of [Ru(bb<sub>7</sub>)(dppz)]<sup>2+</sup>

### Lipophilicity (log *P*)

Lipophilicity is an important factor in the cellular uptake of a compound, particularly for [Ru(N-N)<sub>2</sub>(dppz)]<sup>2+</sup> complexes (where N-N = a bidentate polypyridyl ligand such as bpy, phen, DIP, etc.) that enter cells via passive diffusion.<sup>[14,27]</sup> For example, [Ru(DIP)<sub>2</sub>(dppz)]<sup>2+</sup> (log *P* = +1.30)<sup>[14]</sup> can easily enter cells, whereas, [Ru(phen)<sub>2</sub>(dppz)]<sup>2+</sup> (log *P* = -1.48) cannot. The octanol/water partition coefficients (log *P*) of [Ru(phen)<sub>2</sub>(dppz)]<sup>2+</sup> and [Ru(bb<sub>7</sub>)(dppz)]<sup>2+</sup>, as chloride salts, were measured in water. In agreement with previous studies,<sup>[14]</sup> the log *P* of [Ru(phen)<sub>2</sub>(dppz)]<sup>2+</sup> was determined to be -1.45, while a value of -1.35 was obtained for [Ru(bb<sub>7</sub>)(dppz)]<sup>2+</sup>.

### Antimicrobial activity

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for [Ru(bb<sub>7</sub>)(dppz)]<sup>2+</sup> and [Ru(phen)<sub>2</sub>(dppz)]<sup>2+</sup> against six bacterial strains – a methicillin-resistant *Staphylococcus aureus* (MRSA) and a methicillin-sensitive strain of *S. aureus*; two avian pathogenic (APEC) and uropathogenic (UPEC) strains of *Escherichia coli* and a laboratory strain (*E. coli* MG1655); and *Pseudomonas aeruginosa* (PAO1) – were determined, and the results are summarised in Tables 1 and 2. For comparison, the MIC and MBC values for a selection of antibacterial drugs in clinical use, and included on the WHO list of essential medicines, are also presented. As expected, [Ru(phen)<sub>2</sub>(dppz)]<sup>2+</sup> showed good activity against the Gram-positive bacteria, but poor (64 µg/ml) or no activity (> 128 µg/ml) against the Gram-negative species. Alternatively, [Ru(bb<sub>7</sub>)(dppz)]<sup>2+</sup> displayed good activity against both Gram-positive and Gram-negative bacteria. Against the Gram-positive species, [Ru(bb<sub>7</sub>)(dppz)]<sup>2+</sup> displayed similar activity to [Ru(phen)<sub>2</sub>(dppz)]<sup>2+</sup>, but was at least eight-fold more active against the Gram-negative species. Furthermore, as the MBC values are ≤ double the MIC values, it can be concluded that [Ru(bb<sub>7</sub>)(dppz)]<sup>2+</sup> is bactericidal to both Gram-positive and Gram-negative species. In order to assess the importance of the dppz ligand in [Ru(bb<sub>7</sub>)(dppz)]<sup>2+</sup>, the MIC and MBC values were compared to those previously determined for [Ru(bb<sub>7</sub>)(Me<sub>2</sub>phen)<sub>2</sub>]<sup>2+</sup> (Me<sub>2</sub>phen = 4,7-dimethyl-1,10-phenanthroline) which

has a similar log  $P$  value (-1.41)<sup>[24]</sup> to  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  and  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ . Interestingly,  $[\text{Ru}(\text{bb}_7)(\text{Me}_2\text{phen})_2]^{2+}$  also showed poor activity against the Gram-negative species, suggesting that the dppz ligand is an important aspect of the antimicrobial mechanism of action. As inert polypyridylruthenium(II) complexes have been shown to bind DNA and RNA in live bacteria and eukaryotic cells,<sup>[28,29]</sup> the increased activity of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$ , compared to  $[\text{Ru}(\text{bb}_7)(\text{Me}_2\text{phen})_2]^{2+}$ , is most likely due to the ability of the dppz ligand to bind nucleic acids by intercalation. Although  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  exhibited lower activity against Gram-negative bacteria than gentamicin, its activity profile is similar to that of doxycycline, an antibacterial agent used against a variety of Gram-negative species including *E. coli*.<sup>[30]</sup>

**Table 1.** MIC values ( $\mu\text{g/mL}$ ) for the ruthenium complexes and a selection of clinically used antibacterial agents against Gram-positive and Gram-negative bacterial strains.

Compound	Gram-positive		Gram-negative			
	<i>S.</i>	MRSA	MG1655	APEC	UPEC	PAO1
$[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$	2	2	8	8	8	16
$[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$	4	2	64	64	64	>128
<sup>#</sup> $[\text{Ru}(\text{bb}_7)(\text{Me}_2\text{phen})]^{2+}$	8	8	32	64	128	>128
Gentamicin	0.25	0.25	0.5	0.5	1	0.25
Ampicillin	0.5	4	>128	>128	>128	>128
Doxycycline	4	0.13	16	8	16	32
Ciprofloxacin	1	2	64	64	64	0.5
Vancomycin	0.5	0.5	>128	>128	64	>128

<sup>#</sup>Data for  $[\text{Ru}(\text{bb}_7)(\text{Me}_2\text{phen})]^{2+}$  taken from reference 24



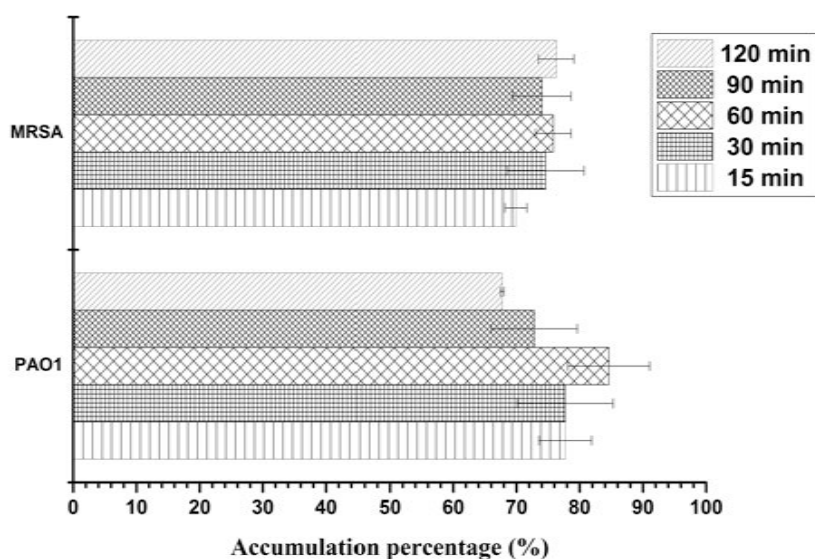
**Table 2.** MBC values ( $\mu\text{g/mL}$ ) for the ruthenium complexes and a selection of clinically used antibacterial agents against Gram-positive and Gram-negative bacterial strains.

Compound	Gram-positive		Gram-negative			
	<i>S. aureus</i>	MRSA	MG1655	APEC	UPEC	PAO1
$[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$	4	4	8	16	16	32
$[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$	8-16	8	64	64	>128	>128
$^{\#}[\text{Ru}(\text{bb}_7)(\text{Me}_2\text{phen})]^{2+}$	64	16	$\geq 128$	$\geq 128$	>128	>128
Gentamicin	2	1	2	1	4	1
Ampicillin	1	4	>128	>128	>128	>128
Doxycycline	1	0.25	32	64	128	32
Ciprofloxacin	2	4	>128	>128	>128	1
Vancomycin	0.5	2	>128	>128	>128	>128

<sup>#</sup>Data for  $[\text{Ru}(\text{bb}_7)(\text{Me}_2\text{phen})]^{2+}$  taken from reference 24

### Cellular accumulation

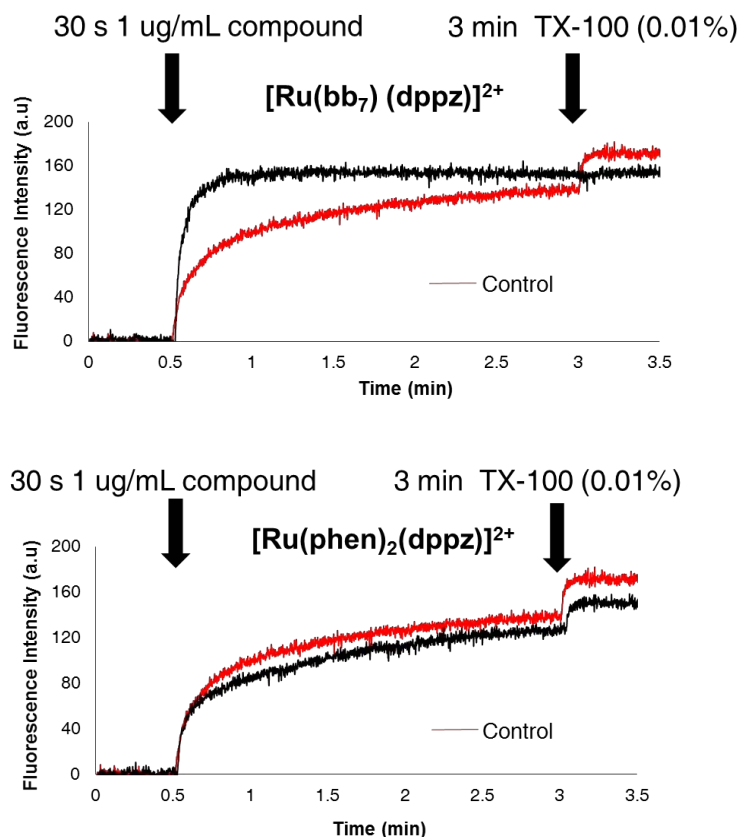
As  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  showed good antimicrobial activity against both Gram-positive and Gram-negative bacteria, it was of interest to compare the cellular accumulation of the ruthenium complex in Gram-positive MRSA and Gram-negative *P. aeruginosa* (PAO1). Although the outer membrane limits the uptake of drugs into the cell for all Gram-negative bacteria, it is particularly significant for *P. aeruginosa*,<sup>[31,32]</sup> which is known to be resistant to many antimicrobials due to the low permeability of its outer membrane. As shown in Figure 2, the cellular accumulation of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  in MRSA and PAO1 are approximately equal, suggesting that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  can be readily taken up by both Gram-positive and Gram-negative bacteria.



**Figure 2.** Cellular accumulation of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  into the Gram-positive bacterium MRSA and Gram-negative PAO1 after incubation for 15, 30, 60, 90 and 120 minutes.

### Liposome membrane permeability

As the cellular accumulation experiments indicated that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  was readily taken up by both Gram-positive and Gram-negative bacteria, its interaction with a model membrane system using carboxyfluorescein-filled 1,2-dioleoylphosphatidylglycerol (DOPG) liposomes was examined. Carboxyfluorescein is a membrane-impermeant fluorescent dye ( $\lambda_{\text{ex}} = 495 \text{ nm}$  and  $\lambda_{\text{em}} = 517 \text{ nm}$ ) that is commonly used in liposomes to examine the effect of potential drugs on membranes.<sup>[33]</sup> Control experiments with colistin, a cationic polypeptide antibiotic known to permeabilise bacterial membranes,<sup>[34]</sup> confirmed the partial release of carboxyfluorescein from the liposomes (see Figure 3), with the remainder of the dye released upon the subsequent addition of Triton-X (a detergent that lyses cells). Upon addition of  $[\text{Ru}(\text{phen}_2)(\text{dppz})]^{2+}$ , the dye release profile was similar to that of colistin (see Figure 3), suggesting that it did have an effect on the permeability of the DOPG liposomes. However, the effect with  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  was much stronger, with all the dye being released very rapidly. Although only a model membrane system, the carboxyfluorescein-filled DOPG liposome experiments suggests  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  has a much stronger effect on membranes than  $[\text{Ru}(\text{phen}_2)(\text{dppz})]^{2+}$ .

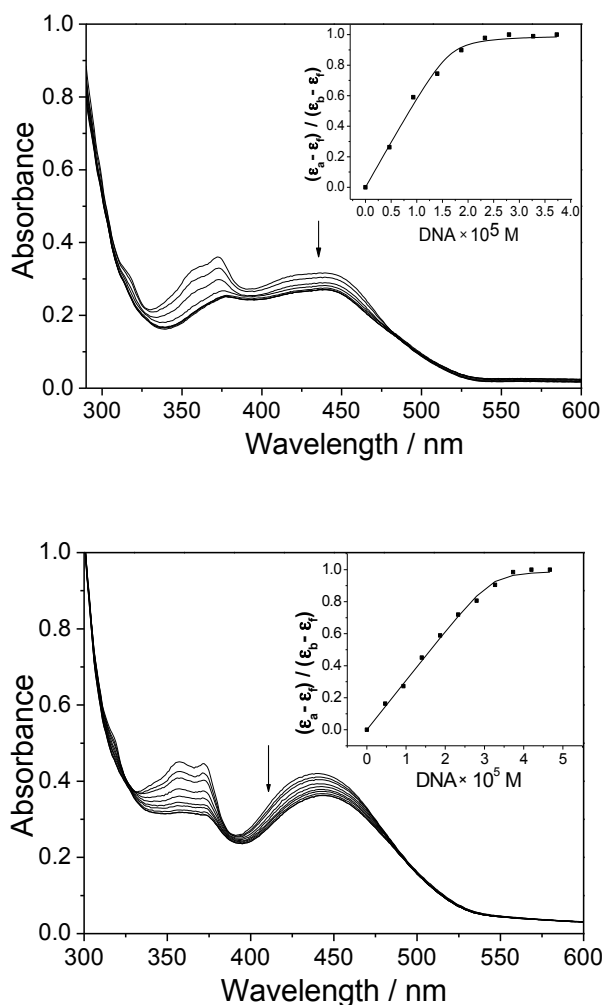


**Figure 3.** The effect of  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  and  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  on DOPG liposomes at  $1 \mu\text{g/mL}$  ruthenium complex concentration. Control (colistin, red curve) and ruthenium complex (black curve).

### DNA binding

The affinities of the  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  and  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  complexes with CT-DNA were determined by UV-visible absorption spectral studies in PBS buffer at room temperature. The absorption spectra of the two complexes in the presence and absence of CT-DNA ( $[\text{Ru}] = 20 \mu\text{M}$ ) are shown in Figure 4. In the visible region, the broad bands at the lowest energy at 439 nm for  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  and  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  are attributed to the metal-to-ligand charge transfer (MLCT) transition. With increasing DNA concentration, the hypochromism in the MLCT band was 12.3% at a ratio of  $[\text{DNA}]/[\text{Ru}] = 2.0$  for  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  and 10.1% at a ratio of  $[\text{DNA}]/[\text{Ru}] = 2.2$  for  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$ . The intrinsic binding constants  $K$  were obtained according to eq.1, and the values determined for  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  and  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  were  $2.6 \times 10^6 \text{ M}^{-1}$  and  $6.7 \times 10^6 \text{ M}^{-1}$ , respectively. The binding constant determined for  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  is consistent with previously reported values by others ( $5.1 \times 10^6 \text{ M}^{-1}$ ).<sup>[35]</sup> From the results obtained, it is clear that the DNA binding affinity of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  is at least equivalent to the parent  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  complex. Furthermore, based upon the significant hypochromic shift observed in the MLCT band at

439 nm, it can be concluded that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  binds DNA by intercalation. As has been demonstrated in many previous studies, the DNA binding constant will be affected to some degree by the nature of the ancillary ligands; consequently, it is not surprising that there is a small difference in affinity between the two dppz-based ruthenium complexes.



**Figure 4.** Absorption spectra of the ruthenium complexes  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  (top) and  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  (bottom) in buffer and upon the addition of CT-DNA,  $[\text{Ru}] = 20 \mu\text{M}$ ,  $[\text{DNA}] = 0 - 50 \mu\text{M}$ . Arrow shows the absorbance changing upon the increase in DNA concentration. Inset: plots of  $(\epsilon_a - \epsilon_f)/(\epsilon_b - \epsilon_f)$  vs.  $[\text{DNA}]$  for the titration of DNA to Ru(II) complexes.

### Toxicity to eukaryotic cells

To further evaluate the potential of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  as an antimicrobial agent, an understanding of its cytotoxicity towards mammalian cells is necessary. The cytotoxicities of  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  and  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  against several eukaryotic cell lines over a 48 hour time-frame were determined, and the results are shown in Table 3. The results indicate

that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  is more toxic than  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  to eukaryotic cells, particularly the HepG2 cancer cell line. However, comparing the  $\text{IC}_{50}$  data to the MIC data ( $1\ \mu\text{M} = 0.9\ \mu\text{g/mL}$ ), it is noted that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  is nevertheless more toxic to the bacteria than to the healthy cell lines HEK-293 and L02.

**Table 3.** 48h- $\text{IC}_{50}$  values ( $\mu\text{M}$ ) of the ruthenium complexes against the eukaryotic cell lines ACHN (kidney cancer cell), HEK-293 (healthy kidney cell), HepG2 (liver cancer cell) and L02 (healthy liver cell).

	ACHN	HEK-293	HepG2	L02
$[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$	$102.9 \pm 30.7$	$103.3 \pm 14.1$	$61.7 \pm 11.1$	$> 200$
$[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$	$22.8 \pm 1.1$	$29.5 \pm 2.6$	$4.9 \pm 0.4$	$71.4 \pm 4.3$
Cisplatin	$2.9 \pm 0.1$	$37.9 \pm 5.9$	$7.4 \pm 1.0$	$14.6 \pm 2.0$

## Discussion

Recently, the WHO published a “Global priority list of antibiotic-resistant bacteria to guide research, discovery and development of new antibiotics”.<sup>[36]</sup> The published priority list was divided into three categories: critical; high; and medium. All the bacteria in the critical class were Gram-negative species, and included *P. aeruginosa* and enterobacteriaceae species (which includes *E. coli*). There is clearly a need for the development of new classes of antimicrobial agents, and kinetically-inert polypyridylruthenium(II) complexes have shown considerable potential in this regard.<sup>[3,4]</sup> A variety of compounds from this class of ruthenium complexes have shown good antimicrobial activities, and recently several have shown good antiparasitic activity in *in vivo* studies.<sup>[37]</sup> However, to date, the much-studied class that contains the dppz ligand has only shown significant activity against Gram-positive bacterial strains.<sup>[21]</sup> In this study, we have shown that incorporating the  $\text{bb}_7$  tetradentate ligand into the  $\text{Ru}(\text{dppz})$  scaffold results in a complex that is active against Gram-negative bacteria, but without increasing the lipophilicity of the complex compared to the parent  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ .

In addition to the differences in antimicrobial activities between the  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  and  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$ , some other interesting differences were observed in the activity profile of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  compared to the previously-reported  $[\text{Ru}(\text{phen})(\text{bb}_7)]^{2+}$  complexes. Although  $[\text{Ru}(\text{bb}_7)(\text{Me}_2\text{phen})]^{2+}$  has an almost identical log *P* value to  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$ , it was much less active than the dppz-based complex to Gram-negative species. This suggests the dppz ligand plays a specific role in the mechanism of antimicrobial activity, and given its well-known role in DNA binding,<sup>[9,10]</sup> it is likely that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  inhibits bacterial

transcription and/or translation processes. DNA binding experiments demonstrated that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  bound CT-DNA slightly more strongly than  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ . Furthermore,  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  exhibited slightly better activity than the more lipophilic  $[\text{Ru}(\text{bb}_7)(\text{Me}_4\text{phen})]^{2+}$ ; surprisingly,  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  was determined to be bactericidal, while  $[\text{Ru}(\text{bb}_7)(\text{Me}_4\text{phen})]^{2+}$  ( $\text{Me}_4\text{phen}$  = 3,4,7,8-tetramethyl-1,10-phenanthroline) was bacteriostatic with very high MBC values ( $> 128 \mu\text{g/mL}$ ) against UPEC and PAO1.<sup>[24]</sup>

It is yet to be determined why  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  displays better activity to Gram-negative bacteria compared to  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ ; however, the results from the DOPG liposome experiments clearly indicate that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  has a greater effect on membrane stability compared to the parent complex. Similar carboxyfluorescein release profiles were observed for  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  and colistin. The polymyxin colistin initially binds by electrostatic interaction between the positively-charged antibiotic and the negatively-charged phosphate group of lipid A on lipopolysaccharide (LPS) localised on the outer leaflet of the bacterial outer membrane.<sup>[34]</sup> Following its diffusion from the outer membrane across the periplasm, the polymyxin intercalates into the inner membrane and forms pores, which in turn results in bacterial lysis. While DOPG liposomes are only a very approximate model of a Gram-negative membrane, it is possible that the ruthenium complexes could also displace  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions from the phosphate groups. Furthermore, as the effect on the DOPG liposomes was significantly stronger and more complete upon addition of  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$ , it is possible that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  either binds more strongly to the membrane (thereby increasing the proportion of the complex partitioning into the membrane) or increases the permeability of the membrane allowing higher concentrations of the ruthenium complex to accumulate in the bacterial cell.

While  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  showed significant activity against Gram-negative bacteria, it is acknowledged that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  is also more toxic towards eukaryotic cells than the parent complex  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ . However,  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  does exhibit greater activity against bacteria than healthy eukaryotic cells. Furthermore, it is likely that the differential between the antimicrobial activity and the toxicity towards eukaryotic cells could be increased through the incorporation of  $\text{bb}_n$  ligands that contain a shorter alkyl chain.

## Conclusions

In summary, the new mononuclear dppz-based complex  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  has been synthesised and characterised. The complex and its parent complex  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$  exhibited high activity against Gram-positive bacteria, but only  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  showed good activity against Gram-negative species. Cellular accumulation assays demonstrated that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  accumulated in Gram-negative

bacteria to the same degree as Gram-positive species. Assays with DOPG liposomes suggested that  $[\text{Ru}(\text{bb}_7)(\text{dppz})]^{2+}$  interacted more strongly with membranes than  $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ , which could make the membranes more permeable to the cationic metal complexes.

## Experimental section

### Materials and methods

All materials were commercially available and were used without further purification. The compounds  $\text{bb}_7$ ,  $\text{dppz}$ ,  $\text{cis-}[\text{RuCl}_2(\text{DMSO})_4]$ ,  $\text{cis,cis-}[\text{RuCl}_2(\text{DMSO})_2(\text{dppz})]$ ,  $\text{cis-}[\text{Ru}(\text{bpy})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O}$  and  $[\text{Ru}(\text{phen})_2(\text{dppz})]\text{Cl}_2$  were synthesised according to the literature methods.<sup>[9,26,38-41]</sup> Amberlite IRA-402 (chloride form) anion-exchange resin and SP-Sephadex C-25 cation exchanger was purchased from GE Health Care Bioscience.

### Physical measurement

$^1\text{H}$  NMR spectra was recorded on a Varian Advance 400 MHz spectrometer in  $\text{d}_6\text{-DMSO}$ . Mass spectra (ESI-MS) were recorded using at the RSC Mass Spectrometry Facility at the Australian National University (Finnigan MAT, USA) and the quoted  $m/z$  values are for the major peaks in the isotope distribution. Emission spectra were recorded on a Cary Eclipse Fluorescence Spectrophotometer at room temperature. Microanalyses (C, H, and N) were performed at the Campbell Microanalytical Laboratory at the Chemistry Department, University of Otago in New Zealand.

### Synthesis of $[\text{Ru}(\text{bb}_7)(\text{dppz})](\text{PF}_6)_2$

A mixture of  $\text{cis,cis-}[\text{RuCl}_2(\text{DMSO})_2(\text{dppz})]$  0.244 g (*ca.* 0.40 mmol),  $\text{bb}_7$  (0.213 g, 0.47 mmol), and ethylene glycol (30 mL) was heated at 130 °C under argon for 2 h. After cooling, the resulting solution was diluted with water (*ca.* 20 mL) and filtered. The filtrate was loaded onto a SP Sephadex C-25 cation-exchange column (3×20 cm). The desired mononuclear complex was eluted with 1.0 M NaCl solution-acetone (5:1, v/v). Solid  $\text{KPF}_6$  was added to the eluate, resulting in the red precipitate of the mononuclear complex. The complex was extracted into dichloromethane ( $2 \times 30$  mL). The organic layer was washed with small amounts of water, then dried over anhydrous  $\text{Na}_2\text{SO}_4$ . Filtration, followed by removal of the solvent and drying of the residue *in vacuo*, gave a red microcrystalline solid. Yield: 0.060 g, 15.3%. Anal. Calcd for  $\text{C}_{47}\text{H}_{42}\text{N}_8\text{F}_{12}\text{P}_2\text{Ru}$ : C, 50.9; H, 3.81; N, 10.1%; Found: C, 51.3; H, 4.00; N, 10.6%. ESI-MS ( $\text{CH}_3\text{CN}$ ):  $m/z = 965.2$  ( $[\text{M-PF}_6]^+$ ), 410.1 ( $[\text{M-2PF}_6]^{2+}$ ).  $^1\text{H}$  NMR (400 MHz, ppm,  $\text{DMSO-d}_6$ ): 9.72 (d,  $J = 8.0$  Hz, 2H;  $\text{dppz}$  4,7), 8.74 (d,  $J = 5.2$  Hz, 2H;  $\text{dppz}$  2,9), 8.54 (dd,  $J_1 = J_2 = 3.2$  Hz, 2H;  $\text{dppz}$  12,13), 8.33 (s, 2H;  $\text{bipy}$  3), 8.19 (m, 2H;  $\text{bipy}$  3'),  $\text{dppz}$  11,14), 7.91 (dd,  $J_1 = J_2 = 5.6$  Hz, 2H;  $\text{dppz}$  3,8), 7.51 (d,  $J = 5.6$  Hz, 2H;  $\text{bipy}$  6), 7.43 (d,  $J = 6.0$  Hz, 2H;  $\text{bipy}$  6'), 7.13 (d,  $J = 5.2$  Hz, 2H;  $\text{bipy}$  5), 7.07 (d,  $J = 5.6$  Hz, 2H;  $\text{bipy}$  5'),

2.86 (m, 2H;  $CH_2$  bipy), 2.67 (m, 2H;  $CH_2$  bipy), 2.50 (s, 6H;  $CH_3$  bipy), 1.67-1.48 (m, 4H;  $CH_2$  bipy), 0.93-0.79 (m, 6H;  $3 \times CH_2$ ). UV/Vis [ $\lambda$ /nm ( $\epsilon/M^{-1} \text{ cm}^{-1}$ )] ( $H_2O$ ): 439 (19200); 372 (22300); 357 (22550); 283 (98900). Luminescence:  $\lambda_{ex}$  = 439 and  $\lambda_{em}$  = 615 nm.

[Ru(bb<sub>7</sub>)(dppz)](PF<sub>6</sub>)<sub>2</sub> was converted to the chloride salt by stirring in methanol with Amberlite IRA-402 (chloride form) anion-exchange resin for 1 h.

### Bacterial strains

Two *S. aureus* (Gram-positive) isolates {a wild type *S. aureus* strain (SH 1000) and a clinical multidrug-resistant MRSA strain (USA 300 LAC JE2)}, and three Gram-negative *E. coli* isolates {MG 1655, NCTC 12241 (APEC) and ST 131 (UPEC)} and a *P. aeruginosa* strain PAO1 (WT), were used for *in vitro* MIC and MBC antimicrobial assays.

### Lipophilicity (log *P*) determination

The measurement of the octanol-water partition coefficients were performed using the “shake-flask” method.<sup>[42]</sup> The chloride salt of the ruthenium complex (0.1 mM) was dissolved in the water phase and an equal volume of *n*-octanol was added. After saturation of both phases by shaking overnight at room temperature, the concentration of the complex in each phase was determined spectroscopically at 450 nm.

### MIC and MBC determination

MIC and MBC were measured by using the broth micro-dilution method described in the CLSI guidelines and the standard microbiological techniques protocol.<sup>[43]</sup> After inoculation with different bacteria with different concentrations of ruthenium complexes, the growth rates of bacteria were determined by counting colony forming unit (cfu) in each plate. The final concentration of bacterial suspension was  $4-8 \times 10^5$  cfu/mL and the final concentration range of ruthenium complexes tested was between 0.25 and 128 mg/L. MICs were recorded after 16–18 h at 37 °C. After incubation for another 4 h, MBCs were recorded when the complexes produced a 99.9% kill relative to the starting inoculum and the wells with no visible growth were taken into colony counting.

### Cellular accumulation

The cellular accumulation of the ruthenium complexes was measured by monitoring the luminescence of the ruthenium(II) complexes that remained in the culture supernatant after removing the bacteria by centrifugation after incubation for various periods of time. Bacterial inocula in the log phase were adjusted to a cell concentration of  $1-5 \times 10^7$  cfu mL<sup>-1</sup>. Cell culture (24 mL) was placed in a 250 mL conical flask and 75  $\mu$ L of stock solution (2.56 mg/mL) of the ruthenium complex was added to give a final concentration of 8  $\mu$ g/mL.



Control flasks containing 50 mL of each bacterial suspension were set up as blank samples to obtain luminescence calibration curves for each complex. Culture flasks and control flasks were incubated with shaking at 200 rpm at 37 °C for 15, 30, 60, 90 or 120 min. At each time point, 3.3 mL of bacterial suspension were centrifuged (5500 rpm) at 4 °C for 10 min. Supernatants (3 mL) were carefully transferred to 5 mL tubes and the luminescence of the remaining ruthenium complex was measured using a Cary Eclipse Fluorescence Spectrophotometer with  $\lambda_{\text{ex}} = 488$  nm and  $\lambda_{\text{em}} = 640$  nm. Volumes (21, 39, 57, 75 and 93  $\mu\text{L}$ ) of a stock solution ( $320 \mu\text{g mL}^{-1}$ ) of each complex were added to 3 mL aliquots of the supernatant from each control bacterial suspension (untreated with drug) to acquire a luminescence-concentration linear correlation chart for calibration.

### DNA-binding Experiments

The DNA-binding experiments were carried out in phosphate-buffered saline (PBS) at pH 7.4. The solution of calf-thymus DNA in PBS buffer gave a ratio of UV absorbance of 1.8-1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The concentration of DNA per nucleotide was determined spectroscopically using a molar absorption coefficient of  $13,300 \text{ M}^{-1} \text{ cm}^{-1}$  per base pair at  $\lambda_{\text{abs}} = 260$  nm.

In order to determine the DNA-binding affinities between complexes and DNA, the absorption titrations of Ru(II) complexes in PBS buffer were carried out by adding an increasing volume of stock DNA solution to the constant concentration (20  $\mu\text{M}$ ) of ruthenium complex solution at room temperature. The complex-DNA solutions were allowed to incubate for 5 min before the absorption spectra were recorded. The intrinsic binding constants  $K$  to DNA were calculated by a non-linear least-square method using eq. 1.<sup>[35]</sup>

$$(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f) = (b - (b^2 - 2K^2C_t[\text{DNA}]/s)^{1/2})/2KC_t \quad (1a)$$

$$b = 1 + KC_t + K[\text{DNA}]/2s \quad (1b)$$

where  $[\text{DNA}]$  is the concentration of DNA in M (nucleotide),  $\varepsilon_a$  is the absorption coefficient observed for the <sup>1</sup>MLCT absorption band at a given DNA concentration,  $\varepsilon_f$  is the absorption coefficient of the free complex without DNA,  $\varepsilon_b$  is the absorption coefficient of the complex fully bound to DNA.  $K$  is the equilibrium binding constant in  $\text{M}^{-1}$ ,  $C_t$  is the total metal complex concentration and  $s$  is the binding site size.

### Liposome leakage experiment

Liposomes were made using 1,2-dioleoylphosphatidylglycerol (DOPG) (sodium salt) according to the literature.<sup>[23]</sup> 5(6)-Carboxyfluorescein, disodium salt (CF, Sigma Aldrich, Taufkirchen, Germany) was enclosed in the aqueous core of the liposomes. Liposome release

assays using carboxyfluorescein were performed as follows: compound sample (1  $\mu\text{g}$  /mL, 20  $\mu\text{L}$ ), liposomes (10  $\mu\text{L}$ ) and buffer {50 mM sodium phosphate, 10 mM NaCl and 1 mM of ethylenediaminetetraacetic acid (EDTA); pH 7.4; 170  $\mu\text{L}$ } were incubated. Fluorescence was measured using a Cary Eclipse Fluorescence Spectrophotometer. Colistin was used as control to determine whether the ruthenium complexes can destroy liposomes and 0.01% Triton X-100 was used to measure complete dye release after incubation with compound for 3 min. For carboxyfluorescein  $\lambda_{\text{ex}} = 490$  nm and  $\lambda_{\text{em}} = 520$  nm.

### Cytotoxicity assays

Half-maximal inhibitory concentrations ( $\text{IC}_{50}$ ) of the ruthenium complexes against ACHN, HEK-293, HepG2 and L02 cell lines were determined using the CCK-8 cytotoxicity assay (Dojindo, Tokyo, Japan) according to the manufacturer's protocol. Cells (3000 per well) were seeded in 96-well plates in RPMI-1640 media with 10% FBS overnight. The cells were treated with various concentrations (ranging between 1.56 and 400  $\mu\text{M}$ ) of the ruthenium complexes for 48 h at 37  $^{\circ}\text{C}$  with 5%  $\text{CO}_2$ . Cisplatin served as an evaluation standard. The average values presented are based on at least three independent experiments. The  $\text{IC}_{50}$  values were determined using GraphPad Prism 6.0.

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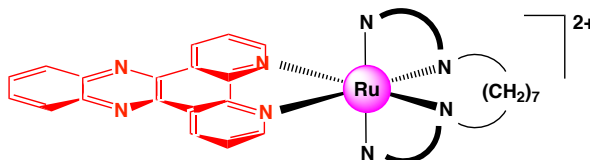
### References

- [1] N. L. Kilah, E. Meggers, *Aust. J. Chem.* **2012**, 65, 1325-1332.
- [2] M. R. Gill and J. A. Thomas, *Chem. Soc. Rev.* **2012**, 41, 3179-3192.
- [3] F. Li, J. G. Collins and F. R. Keene, *Chem. Soc. Rev.* **2015**, 44, 2529-2542.
- [4] H. M. Southam, J. A. Butler, J. A. Chapman, R. K. Poole, *Adv. Microb. Physiol.*, **2017**, 71, 1-96.
- [5] A. Notaro, G. Gasser, *Chem. Soc. Rev.* **2017**, 46, 7317-7337.
- [6] B. M. Zeglis, V. C. Pierre, J. K. Barton, *Chem. Commun.* **2007**, 44, 4565-4579.
- [7] C. A. Puckett, R. J. Ernst, J. K. Barton, *Dalton Trans.* **2010**, 39, 1159-1170.
- [8] G. Li, S. Lingli, L. Ji, H. Chao, *Dalton Trans.* **2016**, 45, 13261-13276.

- [9] A. E. Friedman, J. C. Chambron, J. P. Sauvage, N. J. Turro, J. K. Barton, *J. Am. Chem. Soc.* **1990**, *112*, 4960-4962.
- [10] R. M. Hartshorn, J. K. Barton, *J. Am. Chem. Soc.* **1992**, *114*, 5919-5925.
- [11] A. W. McKinley, P. Lincoln, E. M. Tuite, *Coord. Chem. Rev.* **2011**, *255*, 2676–2692.
- [12] J. A. Smith, M. W. George, J. M. Kelly, *Coord. Chem. Rev.* **2011**, *255*, 2666–2675
- [13] H. van der Salm, A. B. S. Elliott, K. C. Gordon, *Coord. Chem. Rev.* **2015**, *282–283*, 33–49.
- [14] C. A. Puckett, J. K. Barton, *J. Am. Chem. Soc.* **2007**, *129*, 46-47.
- [15] U. Schatzschneider, J. Niesel, I. Ott, R. Gust, H. Alborzinia, S. Wölfl, *ChemMedChem*, **2008**, *3*, 1104-1109.
- [16] J. Hess, H. Huang, A. Kaiser, V. Pierroz, O. Blacque, H. Chao, G. Gasser, *Chem. Eur. J.* **2017**, *23*, 9888-9896.
- [17] V. Pierroz, T. Joshi, A. Leonidova, C. Mari, J. Schur, I. Ott, L. Spiccia, S. Ferrari, G. Gasser, *J. Am. Chem. Soc.* **2012**, *134*, 20376–20387.
- [18] T. Joshi, V. Pierroz, S. Ferrari, G. Gasser, *ChemMedChem* **2014**, *9*, 1419–1427.
- [19] H. Huang, P. Zhang, B. Yu, Y. Chen, J. Wang, L. Ji, H. Chao, *J. Med. Chem.* **2014**, *57*, 8971-8983.
- [20] R. M. Epand, R. F. Epand, *Biochimica et Biophysica Acta* **2009**, *1788*, 289–294.
- [21] A. Bolhuis, L. Hand, J. E. Marshall, A. D. Richards, A. Rodger, J. Aldrich-Wright, *Eur. J. Pharm. Sci.* **2011**, *42*, 313-317.
- [22] F. R. Svensson, M. Matson, M. Li, P. Lincoln, *Biophys. Chem.* **2010**, *149*, 102-106.
- [23] D. K. Weber, M. A. Sani, M. T. Downton, F. Separovic, F. R. Keene, J. G. Collins, *J. Am. Chem. Soc.* **2016**, *138*, 15267-15277.
- [24] B. Sun, H. M. Southam, J. A. Butler, R. K. Poole, A. Burgun, A. Tarzia, F. R. Keene, J. G. Collins, *Dalton Trans.* **2018**, *47*, 2422-2434.
- [25] A. K. Gorle, M. Feterl, J. M. Warner, S. Primrose, C. C. Constantinoiu, F. Richard Keene, J. G. Collins, *Chem. Eur. J.* **2015**, *21*, 10472-10481.
- [26] C. Hiort, P. Lincoln, B. Nordén, *J. Am. Chem. Soc.* **1993**, *115*, 3448-3454.
- [27] C. A. Puckett, J. K. Barton. *Biochemistry* **2008**, *47*, 11711-11716.
- [28] F. Li, E. J. Harry, A. L. Bottomley, M. D. Edstein, G. W. Birrell, C. E. Woodward, F. R. Keene and J. G. Collins, *Chem. Sci.* **2014**, *5*, 685-693.
- [29] X. Li, A. K. Gorle, T. D. Ainsworth, K. Heimann, C. E. Woodward, J. G. Collins and F. R. Keene, *Dalton Trans.* **2015**, *44*, 3594-3603.
- [30] I. Chopra and M. Roberts, *Microbiol. Mol. Biol. Rev.* **2001**, *65*, 232-260.
- [31] Z. Drulis-Kawa, J. Gubernator, A. Dorotkiewicz-Jach, W. Doroskiewicz, A. Kozubek, *Int. J. Pharmaceutics* **2006**, *315*, 59-66.
- [32] H. P. Schweizer, *Expert Opin. Drug Discov.* **2012**, *7*, 633-642.

- [33] M.-A. Sani, E. Gagne, J. D. Gehman, T. C. Whitwell, F. Separovic, *Eur. Biophys. J.* **2014**, *43*, 445-450.
- [34] R. Gao, Y. Hu, Z. Li, Q. Wang, J. Lin, H. Ye, F. Liu, S. Srinivas, B. Zhu, Y.-H. Liu, G.-B. Tian, Y. Feng, *PLoS Pathog.* **2016**, *12*, e10005957.
- [35] R. B. Nair, E. S. Teng, S. L. Kirkland, C. J. Murphy, *Inorg. Chem.* **1998**, *37*, 139-141.
- [36] <http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/>
- [37] M. K. Sundaraneedi, B. A. Tedla, R. M. Eichenberger, L. Becker, M. J. Smout, S. Rajan, P. Wangchuk, F. R. Keene, A. Loukas, J. G. Collins, M. S. Pearson, *PLoS Negl. Trop. Dis.* **2017**, *11*, e0006134.
- [38] Y. Mulyana, D. K. Weber, P. D. Buck, C. A. Motti, J. G. Collins, F. R. Keene, *Dalton Trans.* **2011**, *40*, 1510-1523.
- [39] I. P. Evans, A. Spencer, G. Wilkinson, *J. Chem. Soc. Dalton Trans.* **1973**, 204-209.
- [40] C. S. Burke, T. E. Keyes, RSC. Adv. **2016**, *6*, 40869-40877.
- [41] M.K. Brennaman, T.J. Meyer, J.M. Papanikolas. *J. Phys. Chem. A* **2004**, *108*, 9938-9944.
- [42] F. Lombardo, B. Faller, M. Shalaeva, I. Tetko, S. Tilton, *Molecular Drug Properties: Measurement and Prediction*, Wiley-VCH Verlag GmbH & Co., Weinheim, Germany, **2007**.
- [43] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement M100-S19. CLSI, Wayne, PA, USA, **2009**.

## Graphical Abstract



**Activity against Gram-negative bacteria.** According to the WHO, Gram-negative bacteria pose a major threat to global health. To date, ruthenium(II) complexes containing a dppz ligand (commonly known as “DNA light-switches”) have exhibited poor activity against Gram-negative bacteria. However, incorporation of a tetradentate ligand containing an alkyl chain, as shown in Figure, results in a ruthenium(II)-dppz complex that is active against both Gram-positive and Gram-negative bacteria.

**Keywords:** antimicrobial agents; bioinorganic chemistry; DNA binding; lipophilicity; ruthenium(II) complexes